

Genome Sequence of *Enterococcus hirae* (*Streptococcus faecalis*) ATCC 9790, a Model Organism for the Study of Ion Transport, Bioenergetics, and Copper Homeostasis

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***Enterococcus hirae* ATCC 9790 is a Gram-positive lactic acid bacterium that has been used in basic research for over 4 decades. Here we report the sequence and annotation of the 2.8-Mb genome of *E. hirae* and its endemic 29-kb plasmid pTG9790.**

Enterococcus hirae has been used as a model organism for more than 4 decades. It has been used to address fundamental questions, such as the bioenergetics of phosphate, proton, potassium, sodium, and calcium transport (2, 3, 8, 10, 12, 13, 16, 18), the action of uncouplers and ionophores (5, 6), the identification of penicillin-binding proteins, the mechanism of autolysis (4, 14), or the groundbreaking work on bacterial copper homeostasis (15, 17). Due to the name changes from *Streptococcus faecalis* to *Streptococcus faecium*, and finally to *Enterococcus hirae*, some of the early work with the type strain, ATCC 9790, is difficult to track.

For sequencing, genomic DNA of *E. hirae* was isolated from pure cultures by alkaline lysis (1). Pyrosequencing was carried out with the FLX genome sequencer (Roche, Switzerland). A total of 467,569 shotgun and paired-end reads (3-kb and 8-kb libraries) were assembled with the GS De Novo Assembler version 2.3 (Roche, Switzerland), using the default settings. This resulted in 80 large contigs of an average size of 35,297 nucleotides, with the largest contig encompassing 267,205 bases. The contigs were joined in four scaffolds: 2 repetitive regions, the plasmid pTG9890, and a scaffold with a size of 2,827,741 nucleotides. The 36 contigs comprising the large scaffold had been joined *in silico* and by Sanger sequencing of PCRs spanning the gaps.

The *E. hirae* genome consists of 2,827,741 bases with a GC content of 36.9%. Automatic gene annotation was carried out by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline, which was followed by manual editing. The genome contains 2,813 candidate protein-encoding genes, in addition to 71 tRNA and 18 rRNA genes. The strain harbors an endemic plasmid of 28,699 bp, which we named pTG9790. It encodes 33 hypothetical proteins, of which only four resemble proteins of known function, namely, a UV repair protein, a DNA relaxase, a cysteine protease, and a sporulation initiation protein. (*E. hirae* is not known to sporulate.) Therefore, the function of this plasmid remains largely unknown.

In 1970, Harold et al. described the generation of a sodium-sensitive mutant of *E. hirae* (*S. faecalis*) by chemical mutagenesis (7). In seminal studies, it had served to demonstrate that two different mechanisms exist to extrude sodium: a Na-ATPase and a NaH antiporter (9). Mutant 7683 had later been used to clone the NaH antiporter gene, *napA*, by functional complementation (18). Two spontaneous revertants, R-I and R-II (also R-1 and R-2), which had regained NaH antiport and Na-ATPase activity, respectively, were also described (10, 11). By sequencing the *napA*-encoding region, we could demonstrate a C2240157T mutation (underlined in the sequence GCCAGTAAGCCAACCGAAAA), changing a TGG codon on the reverse coding strand to TGA and

thus changing the W128 codon of *napA* to a stop codon. In revertant R-I, the TGA stop codon was mutated to TCA, resulting in a functional NapA with a W128S mutation. Unfortunately, the mutation inactivating ATP-driven Na extrusion in 7683 could not be identified. In the realm of past and current studies conducted with *E. hirae* ATCC 9790 as a model organism, the present genome sequence should be of great interest.

Nucleotide sequence accession numbers. The genome sequences of *Enterococcus hirae* ATCC 9790 and pTG9790 have been deposited in DDBJ/EMBL/GenBank under accession no. CP003504 and NC_015845, respectively.

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